

1 **Male genital titillators and the intensity of postcopulatory sexual**  
2 **selection across bushcrickets**

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12  
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23

24 **Abstract**

25 Animal genitalia are diverse and a growing body of evidence suggests that they evolve  
26 rapidly under post-copulatory sexual selection. This process is predicted to be more intense  
27 in polyandrous species, although there have been very few comparative studies of the  
28 relationship between the complexity of genital structures in males and measures of the  
29 degree of polyandry. In some bushcricket families, males possess sclerotised copulatory  
30 structures known as titillators, which are inserted into the female's genital chamber and  
31 moved rhythmically. Like other genital structures, bushcricket titillators are widely used as  
32 important taxonomic characters and show considerable variation across species in structure,  
33 shape and the extent to which they are spined. Here, we examine relationships between the  
34 presence/absence of titillators, titillator complexity and both mating frequency and the  
35 degree of polyandry in bushcrickets, using phylogenetic comparative analyses. Using  
36 published sources combined with original observations, data were obtained for the mean  
37 level of polyandry, the duration of the male and female sexual refractory periods and the  
38 level of complexity of titillators. To analyse data, we fitted phylogenetic generalised least  
39 squares models. No significant relationships were found between titillator presence or  
40 complexity and either the level of polyandry, duration of the male's sexual refractory  
41 period or the ratio of the female and male sexual refractory periods. The duration of the  
42 female's refractory period, however, was positively associated with titillator presence and  
43 negatively associated with titillator complexity. The data therefore partially support the  
44 hypothesis that post-copulatory sexual selection drives genital evolution in this taxon.

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47 Genital structures are extremely diverse and are therefore often used as taxonomic  
48 characters to separate closely related species (Eberhard 1985, 2010). Genital traits appear to  
49 diverge more rapidly than non-genital traits (Rowe and Arnqvist 2012). The selective  
50 pressures that drive such rapid evolutionary change in genitalia have been the subject of  
51 considerable debate. These include selection for species isolation (the “Lock and Key”  
52 hypothesis, reviewed in Mikkola 2008), neutral evolution (Pleiotropy hypothesis) and  
53 sexual selection (reviewed in Eberhard 2010; Simmons 2014). The sexual selection  
54 hypothesis potentially involves three closely inter-related processes of post-copulatory  
55 sexual selection: sperm competition, sexually antagonistic co-evolution and cryptic female  
56 choice (Simmons 2014). Strong support for the role of sexual selection in genital evolution  
57 comes from comparative studies that have found a positive relationship between indices of  
58 the degree of polyandry (and hence the intensity of sexual selection) and the degree of  
59 evolutionary divergence or elaboration of genital structures in mammals (Ramm 2007; Orr  
60 and Brennan 2016) and insects (Arnqvist 1998, Rowe and Arnqvist 2012).

61

62 Bushcrickets (Orthoptera: Tettigoniidae) are an excellent model taxon in which to test  
63 hypotheses relating to the evolution of copulatory structures. They are a diverse and  
64 relatively well studied family of insects, which exhibit varying degrees of polyandry  
65 (Gwynne 2001; Vahed 2006, 2007). Two types of sclerotized copulatory structures occur in  
66 male bushcrickets: the cerci and titillators. The cerci act as genital claspers (Rentz 1972,  
67 Vahed et al. 2014). Comparative evidence suggests that sexual conflict over the duration of  
68 ejaculate transfer has affected the form and function of the cerci in bushcrickets (Vahed et  
69 al. 2014, Lehmann et al. 2016). Titillators (Figure 1) are concealed structures that are

70 inserted into the female's genital chamber prior to spermatophore transfer and are moved  
71 rhythmically with contractions of the male's abdomen and phallic complex (Wulff et al.  
72 2015, 2017; Wulff & Lehmann 2016). The tips of the male's titillators contact the sensilla-  
73 rich, un-sclerotised, soft tissues of the opening of the female's genital chamber during  
74 copulation, prior to spermatophore transfer (Wulff et al. 2015, 2017). Like other genital  
75 structures, bushcricket titillators are widely used as important taxonomic characters  
76 (Chamorro-Rengifo and Lopes-Andrade 2014) and show considerable variation across  
77 species in structure, shape and the extent to which they are spined (Vahed et al. 2011).  
78 Comparative evidence indicates that the presence of titillators is associated with longer  
79 copulation durations (prior to spermatophore transfer) in bushcrickets (Vahed et al 2011),  
80 but the relationship between titillator complexity and polyandry has not previously been  
81 examined. In fact, we are not aware of any previous studies that have tested for a  
82 relationship across species between direct measures of the degree of polyandry and genital  
83 complexity in any animal taxon (for studies that have used in-direct measures of the degree  
84 of polyandry or used a binary "monandrous versus polyandrous" classification, see Ramm  
85 2007; Arnqvist 1998; Rowe and Arnqvist 2012; Orr and Brennan 2016; Kuntner et al.  
86 2016). Bushcrickets are one of the few animal groups in which data on the lifetime degree  
87 of polyandry are available for a range of species (Vahed 2006).

88

89 Here, we use phylogenetic comparative analyses to test the prediction that genital  
90 complexity in males will be positively related to the intensity of sexual selection in  
91 bushcrickets. We examine the relationship between titillator complexity and two related  
92 measures of the intensity of sexual selection: the lifetime degree of polyandry and the

93 relative potential reproductive rates (the reciprocal of the sexual refractory period) of males  
94 and females. Where the male's potential reproductive rate exceeds that of the female (i.e.  
95 where the operational sex ratio is male biased), males are assumed to be under stronger  
96 sexual selection (Clutton-Brock & Parker 1992, Ahnesjö et al. 2001: see also Kokko et al.  
97 2014 for a critical review).

98

99 It should be noted that our study is not designed to distinguish between the different  
100 mechanisms of postcopulatory sexual selection (i.e. sperm competition, cryptic female  
101 choice and sexually antagonistic co-evolution). One mechanism of postcopulatory sexual  
102 selection, however, may be distinguishable because it potentially makes the opposite  
103 prediction: If complex genitalia cause damage to the female's reproductive tract (see  
104 Crudginton and Siva-Jothy 2000) and thereby delay the female from re-mating, then more  
105 elaborate genitalia could be associated with a longer "time out" from mating (and therefore  
106 a lower potential reproductive rate in females and a lower degree of polyandry, see  
107 Stockley 2002; Kuntner et al. 2016).

108

109 **Methods**

110 Data on the mean lifetime degree of polyandry were obtained for 29 species of tettigoniid  
111 bushcrickets from published sources including our own studies (Supplementary Table S1).  
112 For the majority of these species, data on polyandry were obtained from counts of  
113 spermatodoses within the spermatheca of tettigoniids sampled at the end of the season in the  
114 field (Gwynne 1984, Vahed 2006, Vahed et al. 2011, Gwynne & Lorch 2013, Kaňuch et al.  
115 2015; Gao Yong, pers. comm.). Spermatodoses are capsules of sperm that are formed within  
116 the female's sperm storage organ (spermatheca) each time she mates (Vahed 2003, 2006,  
117 Parker et al. 2017). Microsatellite analysis of sperm from spermatodoses in the bushcricket  
118 *Pholidoptera griseoptera*, which had mated under natural field conditions, revealed that in  
119 over 80% of females, each spermatodose within the spermatheca was from a different male  
120 (Parker et al. 2017). Re-mating between pairs in bushcrickets is unlikely due to the long  
121 sexual refractory periods of both males and females that occur after each mating, which are  
122 associated with the transfer of the large spermatophores that are typical of this family (Vahed  
123 2007). Because of this, spermatodose counts can be used to estimate the degree of polyandry  
124 in bushcrickets (Gwynne 1984, Vahed 2006, Vahed et al. 2011, Gwynne & Lorch 2013,  
125 Kaňuch et al. 2015). Complementary data were obtained from assessments of caged  
126 populations (in one species, *Lluciapomaresius stalii*, Bateman 1998), or marked field  
127 populations (in six taxa, Heller and von Helversen 1991, McCartney 2010) and, in two  
128 species, molecular analysis of sperm in the spermatheca and offspring of field-mated females  
129 (Hockham et al. 2004, Simmons et al. 2007). Caged populations could yield an exaggerated  
130 degree of polyandry (females may not be able to evade the males as effectively as they would

131 in the field). It should be noted, however, that in the bushcricket species in this study which  
132 was examine in caged populations, *L. stalii*, the males are unable to force the female to  
133 copulate and the females play an active role in pair formation and in mounting the male to  
134 initiate copulation (Bateman 1998). In addition, the degree of polyandry for this species was  
135 at the lower end of the spectrum (mean of 2.1 mates), rather than high. Conversely, studies  
136 of marked individuals in field populations could under-estimate the degree of polyandry  
137 because matings could be missed. The large spermatophore, which remains attached to the  
138 female for several hours after copulation, however, means that matings are unlikely to have  
139 been overlooked in these studies (Heller and von Helversen 1991, McCartney 2010).

140

141 Data on refractory periods for males and females came from individuals assessed for the time  
142 to re-mating in the laboratory (Supplementary Table S1). Individual pairs were assigned to  
143 observation cages at a time of day corresponding to the peak activity period for that species  
144 (see Vahed 2007 for further details). Data from two Australian species were from mating  
145 observations in field cages (Lehmann 2007, Lehmann and Lehmann 2007). These refractory  
146 periods correspond with ‘time-outs from matings’ and are therefore tightly linked with the  
147 concept of potential reproductive rate ( $= 1/\text{time-out}$ , Clutton-Brock & Parker 1992, Ahnesjö  
148 et al. 2001).

149

## 150 **Titillator structure**

151 Data on titillator morphology (Figure 1) were obtained from taxonomic sources, chiefly Harz  
152 (1969) for the majority of European species (Supplementary Table S1). We developed a  
153 ranked classification system to reflect titillator complexity, with emphasis on the complexity

154 (e.g. presence/absence of visible spines; clumping of spines at the tip; single or double pair  
155 of projections) of the apical part of the titillators (median projection) which makes contact  
156 with the female during copulation (Wulff et al. 2015, 2017) (see Figure 1, Table 1). Titillators  
157 were scored blind (by both the first and the last authors) for a previous study (Vahed et al.  
158 2011), i.e. without knowledge of polyandry or duration of male or female refractory periods.

159

## 160 **Analysis**

161 We split “titillator complexity” into two components analysed in parallel: presence/absence  
162 of titillators, analysed as a binary variable (henceforth titillator presence), and complexity of  
163 titillators where present (henceforth titillator complexity), analysed as a continuous variable.  
164 This is because it would make little sense to treat species without titillators effectively as  
165 bearing “titillators of zero complexity”.

166

167 All analyses were conducted in R 3.2.0 (R Core Team 2015). To account for non-  
168 independence due to phylogenetic relatedness, we used comparative-phylogenetic statistical  
169 methods that account for this non-independence by modelling errors in the model residuals  
170 as a function of the phylogenetic distance between species and the underlying model of how  
171 traits evolve along branches. We analysed continuous data (refractory periods) using  
172 phylogenetic generalized least squares models (PGLS; Grafen, 1989, Martins and Hansen,  
173 1997) using the `ppls()` function in the *caper* package (Orme et al. 2013), simultaneously  
174 estimating Pagel's  $\lambda$ , i.e. how closely the tree structure can predict covariance among the  
175 model's residuals (Pagel 1999, Freckleton et al. 2002). For analysis of binary or Poisson data



176 (titillator presence/absence and polyandry, respectively) we fitted Generalised Estimating  
177 Equation (GEE) models (Paradis & Claude 2002), which are more appropriate for non-  
178 normally distributed data (Paradis 2006), using the `compar.gee()` function in the *ape* package  
179 (Paradis et al 2004). We used standard residual plots to assess model fit by eye.

180

181 We initially tested whether, within our dataset, polyandry was associated with the duration  
182 of the female or male refractory periods and whether female and male refractory periods were  
183 associated, as would be predicted (Vahed 2006, 2007). We first fitted a PGLS model of  
184 polyandry with female or male refractory period as a predictor variable, then a PGLS model  
185 with female refractory period as a response and male refractory period as a predictor. Data  
186 on polyandry were log-transformed before analysis to improve model fit. Then, for each  
187 analysis of titillator structure (binary GEE models of presence; continuous PGLS models of  
188 complexity) we fitted models against predictor variables including polyandry, female  
189 refractory period and male refractory period. We performed analyses of potential  
190 reproductive rate directly on male and female reproductive rate, as this improved model fit.  
191 We additionally ran analyses including the reciprocal of the ratio of the male and female  
192 refractory period as a predictor variable. This represents the relative reproductive rates of  
193 males and females (a measure of the operational sex ratio).

194

195 To avoid confounds due to variable measuring techniques, we re-ran all analyses excluding  
196 species where polyandry was assessed using molecular rather than field-based methods (*R.*  
197 *verticalis*, *E. ephippiger*) or where field rather than lab-based methods were used to assess

198 the male refractory period (*P. affinis*, *P. v. veluchianus*) or the female refractory period (*P.*  
199 *affinis*, *P. v. veluchianus*, *P. v. minor*). See Supplementary Table S1 for details.

200

201 The phylogeny used for all analyses was based primarily on the molecular phylogeny by  
202 Mugleston et al. (2013) supplemented by the molecular phylogenetic information derived  
203 from barcoding of Central European species (Hawlitschek et al. 2016), combined with the  
204 morphological phylogeny developed by Naskrecki (2000) (this phylogeny did not use  
205 titillators as a character). For the sub-family Tettigoniinae, we used the morphological  
206 phylogeny provided by Rentz and Coless (1990) (majority consensus tree of 50 equally short  
207 cladograms) because many of the genera were neither included in Mugleston et al. (2013)  
208 nor in Naskrecki (2000). For phylogenetic relationships within the genus *Anonconotus*  
209 (Tettigoniinae), we used an unpublished molecular phylogeny based on mtDNA (R. Szabo,  
210 G. Carron, K. Vahed & M. Ritchie). For the genus *Poecilimon* (Phaneropterinae), we used  
211 the molecular phylogeny given in Ullrich et al. (2010), for the *Poecilimon propinquus*-group  
212 the mtDNA tree of Lehmann (1998). Branch lengths were not available and so we ran all  
213 analyses twice, arbitrarily scaling branches according to node depth, following Grafen  
214 (1989), or setting all branch lengths to 1. We conducted our analyses on the datasets for which  
215 relevant data were available (n=48). For analyses involving subsets of the full dataset, the  
216 full tree was pruned to the appropriate set of taxa only after arbitrary branch lengths had been  
217 assigned, in order to preserve the node depths in the full tree. The datasets supporting this  
218 article have been uploaded as Supplementary Table S1.

219

## 220 **Results**

221 Our analyses returned similar results regardless of whether we excluded minority methods,  
222 or how we assigned branch lengths (arbitrarily to 1 or scaling according to node depth). Thus,  
223 we present only results using all available data regardless of the method used to obtain them,  
224 and using Grafen's (1989) node-depth scaling to assign arbitrary branch lengths to the tree.

225

### 226 *Polyandry*

227 There was a high variation in polyandry in bushcricket females, ranging from as low as a  
228 mean of 1.5 matings up to a mean of 25 to 28 matings per female in *Anonconotus* species  
229 (Figure 2). The data were largely biased towards species with relatively low polyandry, i.e.  
230 between 1.5 and 3.1 numbers of matings over the female lifespan. Polyandry was  
231 significantly negatively associated with the duration of the female refractory period (GEE,  
232  $df=1$ ,  $P=0.013$ ,  $n=12$ ) and the male refractory period (GEE,  $df=1$ ,  $P=0.047$ ,  $n=14$ ). Female  
233 and male refractory periods were positively associated with each other ( $F_{1,25}=18.23$ ,  $p<0.001$ ,  
234  $n=27$ ); in this model Pagel's  $\lambda$  had wide confidence intervals (0 to 0.906), indicating low  
235 certainty in the estimate of phylogenetic signal.

236

237 In the full dataset, titillators were absent in 15 species and present in 32; titillator complexity  
238 in species with titillators was distributed as shown in Figure 2. Polyandry was related neither  
239 to titillator presence (PGLS:  $F_{1, 27}=0.0142$ ,  $P=0.91$ ,  $n=29$ ; Figure 3a) nor to titillator  
240 complexity (PGLS,  $F_{1, 20}=2.56$ ,  $P=0.13$ ,  $n=22$ ; Figure 3b) and vice versa: polyandry was a  
241 good predictor neither of titillator presence (GEE,  $df=1$ ,  $p=0.98$ ,  $n=29$ ) nor complexity

242 (PGLS,  $F_{1, 20}=2.56$ ,  $P=0.13$ ,  $n=22$ ). Estimates of Pagel's  $\lambda$  ranged from 0.94 to 1.00 [95%  
243 CIs  $\sim 0.7 - 1$ ] for these models, indicating a high degree of phylogenetic signal, i.e. covariance  
244 in model residuals could be predicted by phylogenetic distance.

245

#### 246 *Male refractory period*

247 The male refractory period can span over several days, the most extreme mean data coming  
248 from *Antaxius hispanicus* with 7 days, and *Poecilimon thessalicus* with 6 days. In contrast,  
249 several species are able to re-mate within an hour down to a few minutes. Titillator presence  
250 did not reliably depend on the male refractory period (PGLS:  $F_{1, 30} = 0.51$ ,  $P = 0.41$ ,  $n=32$ ,  
251 Figure 3c) but titillator complexity showed a marginal trend suggesting that species with  
252 longer male refractory periods have more complex titillators (PGLS:  $F_{1,18}=3.71$ ,  $P=0.069$ ,  
253  $n=20$ , Figure 3d). The phylogenetic signal estimate had wide confidence intervals ( $\lambda\sim 0.81$   
254 [CI 0.28, 0.96]). The male refractory period did not predict titillator presence (GEE:  $df=1$ ,  
255  $P=0.49$ ,  $n=32$ ) but again showed a marginally positive association with titillator complexity  
256 (PGLS:  $F_{1, 18}=3.66$ ,  $P=0.072$ ,  $n=20$ ,  $\lambda=0.77$ [CI 0.33, 0.95]).

257

#### 258 *Female refractory period*

259 The female refractory period in most species was typically longer than that of the male,  
260 ranging from 1.07 h to 19 days. Unlike the male refractory period, the female refractory  
261 period was positively associated with titillator presence (PGLS:  $F_{1, 26}=9.04$ ,  $P=0.005$ ,  $n=28$ ;  
262  $\lambda=0.62$ , Figure 3e) and negatively associated with titillator complexity (PGLS:  $F_{1,13}=18.052$ ,  
263  $n=15$ ,  $P<0.001$ ;  $\lambda=0$ , Figure 3f). Analysing the inverse relationships, the female refractory

264 period did not predict titillator presence (GEE,  $df=1$ ,  $P=0.15$ ,  $n=28$ ) but was a good predictor  
265 of titillator complexity ( $F_{1,13}=18.052$ ,  $P<0.001$ ,  $n=15$ ;  $\lambda=0$ ).

266

267 *Male vs female potential reproductive rates*

268 The Operational Sex Ratio (measured as the reciprocal of the ratio of female and male  
269 refractory periods) was not associated with titillator presence (PGLS:  $F_{1,25}=1.164$ ,  $P=0.16$ ,  
270  $n=27$ ,  $\lambda=0.85$ ; Figure 3g) nor with titillator complexity (PGLS:  $F_{1,13}=0.94$ ,  $P=0.35$ ,  $n=15$ ;  
271  $\lambda=0.60$ ; Figure 3h). The inverse relationships were similarly nonsignificant, as the  
272 Operational Sex Ratio predicted neither titillator presence (GEE:  $df=1$ ,  $P=0.73$ ,  $n=27$ ) nor  
273 complexity (PGLS:  $F_{1,13}=1.00$ ,  $P=0.34$ ,  $n=15$ ;  $\lambda=0.66$ ).

274

275

276 **Discussion**

277 No significant relationships were found between titillator complexity and any of the degree  
278 of polyandry in bushcrickets, the duration of the sexual refractory period in males, or the  
279 relative potential reproductive rates of males and females. The duration of the female's sexual  
280 refractory period, however, was associated with both titillator presence and complexity. The  
281 results, therefore, provide partial support for the hypothesis that post-copulatory sexual  
282 selection is a driving force in titillator evolution. It should be noted, however, that while there  
283 was a high degree of variation between species in the degree of polyandry (ranging from a  
284 mean of 1.5 to a mean of 28 matings per lifetime), there were no entirely monandrous species  
285 in this data set. Any effect of post-copulatory sexual selection on genital evolution should be  
286 most detectable by comparing monandrous with polyandrous species (Arnqvist 1998). On  
287 the other hand, previous studies have found evidence for a positive relationship between  
288 indicators of the degree of polyandry, measured on a continuous scale, and the complexity or  
289 degree of development of the males' genitalia in both insects and mammals (Ramm 2007,  
290 Rowe and Arnqvist 2012, Orr and Brennan 2016).

291

292 The duration of the female's sexual refractory period was significantly longer in species in  
293 which titillators were present. On the face of it, this appears to be opposite to the prediction  
294 of the sexual selection hypothesis, which predicts that titillators should be more complex in  
295 species in which females are more polyandrous (and therefore have *shorter* sexual refractory  
296 periods, since there was a significant negative relationship between the duration of the  
297 female's sexual refractory period and the lifetime degree of polyandry in our present study).

298 The results are, however, consistent with the hypothesis that complex genitalia-delay or deter  
299 the female from remating, which can be driven by various mechanisms of post-copulatory  
300 sexual selection. Stockley (2002), for example, found that in primates, relatively high penile  
301 spinosity was associated with lower potential reproductive rates in females and interpreted  
302 this in terms of internal damage caused to the female by the spines. Kuntner et al. (2016)  
303 similarly found that in nephilid spiders, the male's genitalia were more complex in  
304 polyandrous species than in monandrous species. Titillators in some bushcrickets have spines  
305 (see Figure 1) that contact the soft, un-sclerotised lining of the female's bursa copulatrix  
306 (Wulff et al. 2015, 2017). However, we found no evidence for any damage by the titillators  
307 to the female's reproductive tract (Wulff et al. 2015, 2017; Wulff and Lehmann 2016), in  
308 contrast to the action of penile spines in *Callosobruchus* beetles (Hotzy and Arnqvist 2009).  
309 Comparative evidence suggests that genital damage selects for females to evolve a thicker  
310 wall of the bursa copulatrix to minimise damage by the male's spines in seed beetles  
311 (Coleoptera: Bruchidae) (Rönn et al. 2007). The thickness of the parts of the female contacted  
312 by the male's genitalia was not measured in the present study. There is strong evidence from  
313 other comparative studies of other insect taxa that co-evolution between male and female  
314 genital structures does occur (Rönn et al. 2007, Yassin and Orgogozo 2013, reviewed in  
315 Simmons 2014) but for several reasons female genitalia are still understudied (Ah-King et  
316 al. 2014, Brennan and Prum, 2015) even in bushcrickets.

317

318 Amongst species with titillators, shorter sexual refractory periods were associated with more  
319 complex titillators. These results appear to be in contrast to the analysis based on the  
320 presence/absence of titillators (see above). Shorter sexual refractory periods were associated

321 with a higher degree of polyandry (this study), so may be used as an indicator of the degree  
322 of polyandry. Comparative studies of water-striders (Heteroptera: Gerridae) have similarly  
323 found relationships between indices of the degree of polyandry and genital complexity (Rowe  
324 and Arnqvist 2012). The results of the present study should be interpreted with caution,  
325 however. The relationship between the duration of the female's sexual refractory period and  
326 titillator complexity appear to be driven by the two species in the sub-family Zaprochilinae  
327 in our dataset, which have unusually long female sexual refractory periods (Simmons and  
328 Gwynne 1991; Lehmann and Lehmann 2007) and simple titillators (Rentz, 1993). This  
329 subfamily is phylogenetically distinct from the majority of other Tettigoniid families. It  
330 should also be noted that we did not find a significant relationship between the actual degree  
331 of polyandry itself (rather than the female's sexual refractory period) and titillator complexity  
332 in the present study (the two analyses were based on slightly different subsets of species, see  
333 Figure 2).

334

335 It is possible that the classification scheme of titillators used in the present study does not  
336 reflect adequately the characters of the titillators that are subject to sexual selection. An  
337 alternative approach would be to use more complex morphometric analyses to quantify the  
338 shape and/or to measure the relative sizes of parts of the titillators (see, for example Rowe  
339 and Arnqvist 2012). In addition, denser sampling of species within selected titillator-  
340 possessing genera would allow for a more fine-scale examination of the relationship between  
341 titillator morphology and polyandry. In the present study, most titillator-possessing genera  
342 were represented by only three species or fewer.

343



344 Although the present study provided mixed support for sexual selection hypotheses for  
345 genital evolution, experimental manipulations, in which either one or both titillators were  
346 removed, have provided some support for the role of cryptic female choice (Wulff et al. 2015,  
347 Vahed 2015, Wulff and Lehmann 2016). The results of these manipulations indicated that  
348 correct stimulation by titillators is necessary for the proper insertion of the spermatophore  
349 and to avoid resistance by females during copulation (Wulff et al. 2015, 2017; Wulff and  
350 Lehmann 2016). Such results, however, are also consistent with the “Lock and Key”  
351 hypothesis, a hypothesis that was not tested in the present study. Simmons (2014) pointed  
352 out that female choice that enforces species isolation and female choice that targets variation  
353 in male quality within populations may be seen as part of the same continuum.

354 To conclude, the present comparative study provided only partial support for the hypothesis  
355 that post-copulatory sexual selection has driven the evolution of titillator complexity in  
356 bushcrickets. The inclusion of monandrous species in the sample, examination of the parts  
357 of the female’s reproductive tract that are contacted by the titillators and denser sampling of  
358 selected genera, however, would be necessary to test the sexual selection hypotheses more  
359 fully.

360

361

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365

366

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537 **Figure legends**

538 **Figure 1:** Left hand side: Electron micrograph of Titillator tips with spines in the tettigoniid  
539 bushcricket *Metrioptera saussuriana*; Right hand side: Variation in titillator morphology in  
540 the Tettigoniidae. The numbers represent the system of titillator classification used in the  
541 present study (see Table 1). Images adapted from Rentz and Birchim (1968) and Rentz  
542 (1993). Note that titillator types 2 and 3 are shown together because they are similar in overall  
543 structure (although the apical part that contacts the female, the median projection, is much  
544 less strongly projecting in type 2 than in type 3).

545

546 **Figure 2:** Variation of male and female potential reproductive rate (reciprocal of the sexual  
547 refractory period), their ratio (female potential reproductive rate divided by the male potential  
548 reproductive rate) and lifetime degree of polyandry, across the bushcricket phylogeny. Data  
549 have been scaled such that open and closed circles represent the minimum and maximum in  
550 the dataset, respectively. For raw data, see Supplementary Table S1.

551

552 **Figure 3:** a) Lifetime polyandry in bushcricket species lacking titillators (“No”) and with  
553 titillators (“Yes”); b) Polyandry against titillator complexity (ordinal ranked scale, see Table  
554 1); c) The duration of the male sexual refractory period in bushcricket species lacking  
555 titillators (“No”) and with titillators (“Yes”); d) The duration of the male sexual refractory  
556 period against titillator complexity; e) The duration of the female sexual refractory period in  
557 bushcricket species lacking titillators (“No”) and with titillators (“Yes”); f) The duration of  
558 the female sexual refractory period against titillator complexity; g) Operational sex ratio

559 (measured as the reciprocal of the ratio of the female and male refractory periods) in  
560 bushcricket species lacking titillators (“No”) and with titillators (“Yes”); h) Operational sex  
561 ratio against titillator complexity.

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563

564 **Table 1:** The titillator classification scheme used in this study (adapted from Vahed et al.  
 565 2011).  
 566

Numerical classification	Explanation	Examples
0	Titillators absent	<i>Poecilimon</i>
1	No sclerotised titillators, but a densely covered field of small tubercles	<i>Kawanaphila</i>
2	One pair of sclerotised titillators, apical part (median projection) conical and not strongly projecting, may have minute teeth	<i>Ruspolia, Yersinella</i>
3	One pair of sclerotised titillators: apical part strongly projecting, with no teeth (the tip however can be hooked)	<i>Metrioptera roeselii</i>
4	One pair of sclerotised titillators: apical part strongly projecting with clearly visible teeth	<i>Anonconotus, Decticus</i>
5	One pair of sclerotised titillators: apical part strongly projecting with teeth concentrated on the club shaped tip	<i>Metrioptera saussuriana</i>
6	Two pairs of sclerotised titillators: apical part strongly projecting with teeth on one or both pairs.	<i>Gampsocleis, Antaxius</i>

567

568

569 **Supplementary Table S1:** Data base for levels of titillator complexity, polyandry and male  
 570 and female refractory periods.